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Cross-talk between Rho GTPases and PI3K in the Neutrophil

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Abstract

Neutrophils are short-lived, abundant peripheral blood leukocytes that provide a first line of defense against bacterial and fungal infections whilst also being a key part of the inflammatory response. Chemokines induce neutrophil recruitment to inflammatory sites, where neutrophils perform a number of diverse functions that are aimed at fighting infections. Neutrophil effector functions are tightly regulated processes that are governed by an array of intracellular signaling pathways and initiated by receptor-ligand binding events. Dysregulated, neutrophil activation can result in excessive inflammation and host damage, as is evident in a number of autoimmune diseases. Rho family small GTPases and agonist-activated phosphoinositide 3-kinases (PI3Ks) represent two classes of key regulators of the highly specialized neutrophil. Here we review cross-talk between these important signaling intermediates in the context of neutrophil functions. We include PI3K-dependent activation of Rho family small GTPases and of their guanine nucleotide exchange factors and GTPase activating proteins, as well as Rho GTPase-dependent regulation of PI3K.

Introduction

Neutrophils are the most abundant peripheral blood leukocytes in humans. These terminally differentiated, highly specialized phagocytes represent a first line of defense against bacterial and fungal infections.[1, 2] As part of the innate immune system, neutrophils do not distinguish between host and intruder but employ their destructive force indiscriminately. Neutrophils need to be tightly controlled. Insufficient neutrophil activity renders the host susceptible to repeated infections, which can be life-threatening. Yet, dysregulated neutrophil activation can result in excessive collateral damage to the host, as exemplified by a range of autoimmune diseases, such as rheumatoid arthritis.

Upon activating conditions, adhesion molecules (selectins and integrins) undergo changes that permit the circulating neutrophil to interact with the vessel wall. Neutrophils roll along the vessel wall, slow down and crawl along the inside of the vessel until they adhere firmly and extravasate. Following gradients of chemokines and chemoattractants neutrophils travel through tissue to reach sites of infection or insult. Once arrived at their destination, neutrophils phagocytose pathogens. They degranulate, releasing an arsenal of cytotoxic enzymes and generate reactive oxygen species (ROS) to kill pathogens (Figure 1). Moreover, neutrophils can release their chromatin to act as extracellular traps (NETs), for example aiding the destruction of pathogens that are too large to be engulfed. Neutrophils also produce cytokines, to recruit and cross-talk with other immune cells thus playing a part in orchestrating the immune response. Circulating neutrophils are only short-lived, with estimated half-lives ranging from hours to days depending on the method employed. Neutrophil lifespan is, however, significantly extended under inflammatory conditions. To end their short lives, aged circulating neutrophils home to liver, spleen and bone marrow for clearance by resident macrophages. The majority of the longer-lived post-migrated, inflammatory neutrophils eventually undergo apoptosis, and display ‘eat-me’ signals. By doing so they, too, induce their own clearance by phagocytic macrophages in a process termed efferocytosis. In this way, neutrophil apoptosis helps to keep the fine balance between the generation and resolution of inflammation, enabling immunity whilst avoiding excessive inflammation.

Ligation of neutrophil cell surface receptors triggers a large number of signaling pathways, that are involved in the tight control of neutrophil behavior. Key regulators include Rho family small GTPases as well as agonist-activated PI3Ks.

Small GTPases cycle between a GDP- and a GTP-bound form; only when GTP-bound can they interact with and activate effector molecules. Small GTPases are themselves subject to regulation. The intrinsic GTPase activity of many GTPases is low; it is activated by GTPase activating proteins (GAPs), whilst exchange of GDP for GTP is catalyzed by guanine nucleotide exchange factors (GEFs). A third regulator, Rho-GDP dissociation inhibitor (RhoGDI) prevents GTP exchange and sequesters the GTPase in the cytosol, thereby aiding the spatial regulation of Rho GTPase signaling (reviewed in ref [3]). Major Rho family small GTPases expressed by human neutrophils are Rac proteins (Rac1, Rac2 and RhoG), RhoA and Cdc42, with several other Rho GTPases expressed at minor levels.[4] Rho GTPases are best known for their regulatory function in dynamic actin rearrangements, but they also control a host of other cellular functions by regulating several effector proteins each.

Few reliable, cell permeable drugs for small GTPases, and their regulators have been developed yet, and primary neutrophils are not amenable to culture, transfection or transduction. Most of our current understanding of small GTPases and of their regulators in the neutrophil therefore stems from genetically manipulated mice, or from (human) leukemia cell lines that can be induced to become neutrophil-like. These models have shown that most if not all neutrophil functions are subject to regulation by Rho family small GTPases and their GEFs and GAPs (see table 1 for some examples). Rho GTPase are particularly involved during neutrophil recruitment, which includes several distinct steps that are themselves Rho GTPase-dependent, e.g. polarization, transendothelial migration (TEM) and chemotaxis. These processes have been the subject of particularly thorough investigation by many groups. A second important regulatory contribution to neutrophil function is found during microbial killing, which again comprising distinct processes that are subject to regulation by Rho GTPases, e.g. phagocytosis, the NADPH oxidase and degranulation.

Class I (also known as agonist-activated) phosphoinositide 3-kinases (PI3Ks) represent a second class of key regulators in the neutrophil.[5] These PI3Ks phosphorylate the membrane lipid phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] in the D3 position to generate the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3). PI3Ks are heterodimeric enzymes that consist of a catalytic subunit (p110α,β,δ; class IA or p110γ; class IB) and a regulatory subunit (a p85/p55-style adaptor for class IA, and a p101 or p84 adaptor for class IB PI3Ks). PI3Kα and δ act downstream of receptor tyrosine kinases (RTK; with phosphopeptide binding of the p85 adaptor). PI3Kγ is activated downstream of G protein coupled receptors (GPCRs) by G protein βγ subunits, and PI3Kβ is synergistically activated by both phosphopeptide and Gβγ. In addition, PI3Ks are subject to regulation by Ras proteins, with GTP-bound Ras binding to the Ras binding domain (RBD) of p110α,δ and γ whilst GTP-bound Rac or Cdc42 can activate p110β by binding to its RBD.[6] Whilst PI3Kα and PI3Kβ are ubiquitously expressed, whilst the vast majority of PI3Kδ and PI3Kγ is expressed in leukocytes. Neutrophils express all four PI3K isotypes. The analysis of isotype usage in any biological scenario is aided by the availability of mouse knock-outs and catalytic dead knock-ins, as well as the development of isoform-selective PI3K inhibitors. The analysis of PI3K isoforms in N-Formyl-methionyl-leucyl-phenylalanine (fMLF)-stimulated mouse and human neutrophils has revealed significant cross-talk between isoforms following neutrophil stimulation as well as distinct differences between signaling pathways in mouse and human neutrophils. [7, 8]

Agonist-activated PI3Ks signal through multiple effector proteins, with an average cell estimated to express between 25 and 50 PI3K effectors. Such effectors include enzymes and adapters, that are activated catalytically and/or recruited to the plasma membrane by PIP_3. A notable fraction of PI3K effectors in the neutrophil comprises regulators of small GTPases,[9] indicative of the large amount of cross-talk between these two classes of regulators. Here, we discuss cross-talk between PI3K and Rho family small GTPases in the neutrophil. There are numerous examples of Rho GTPase-dependent regulation of neutrophil function, involving PI3K-regulated GEFs and GAPs. As indicated, many studies analyzed this in the context of neutrophil polarization and chemotaxis. PI3K (in conjunction with Rac) was thought to be a key to the neutrophil's 'chemotactic compass' for some time. However, this view has since been revised and PI3K's role in chemotaxis is now thought to be context-dependent.[5, 10]

Rac

Rac is best known as the regulator of actin polymerization (Arp2/3-dependent, branched meshworks) as found in actin-rich lamellipodia, which are very important for neutrophil function. Neutrophils express the leukocyte-enriched Rac2 as well as the ubiquitous Rac1 and the Rac-related RhoG. Rac proteins have been extensively studied in neutrophils, in particular genetically in the mouse. To summarize, both Rac1 and Rac2 have important functions in the neutrophil (e.g. refs [11-13]). Much of this data is not very recent; since it has been extensively reviewed previously, it is not covered in-depth here. In-line with the well-documented function of Rac in the NADPH oxidase in other cell types, the neutrophil's NADPH oxidase function was found to be dramatically reduced in Rac2-deficient neutrophils. Interestingly, it was not affected by Rac1-deficiency. Similarly, phagocytosis was reduced by Rac2- but not by Rac1-deficiency. In contrast, both Rac isoforms were reported to be involved in chemoattractant-induced dynamic actin rearrangements and were required for efficient transwell chemotaxis *in vitro*. In keeping with this, lack of either Rac isoform interfered with efficient neutrophil recruitment to sites of inflammation *in vivo.* Analysis of chemotactic tracks *in vitro* suggested that Rac1 regulated chemotactic directionality whilst Rac2 was required for neutrophil migration and speed. RhoG, which functions upstream of Rac1/2,[14] regulates the NADPH oxidase following GPCR stimulation, but it was shown to be dispensible for transwell chemotaxis or neutrophil recruitment *in vivo.*[15]

PI3K-dependent regulation of Rac

A strong link between PI3K and Rac activation has been observed in many contexts. Since it was established that Rac activation can be PI3K-dependent, a number of PIP_3-activated Rac GEFs have been described (reviewed in ref [16]). Several of these, e.g. Vav, P-Rex, DOCK and Tiam GEFs, are expressed in the neutrophil, and neutrophils from some relevant mouse knock-out lines have been analyzed. Vav GEFs were shown genetically to regulate integrin dependent processes,[17] whilst P-Rex1 regulated GPCR-dependent processes such as ROS production.[18] Individually, neither of these GEFs were found to be major regulators of neutrophil migration. In combination, however, Vav1/3 and P-Rex1 deletion significantly impaired neutrophil chemotaxis *in vitro* and neutrophil recruitment to sites of sterile inflammation *in vivo*.[19, 20] Contrasting with the mild chemotaxis defects of Vav1/3 and P-Rex1-deficient neutrophils, loss of Tiam2 (expression of which is abrogated in ATF3 transcription factor knock-out neutrophils) has been shown to interfere with neutrophil chemotaxis *in vitro* and recruitment to the inflamed lung *in vivo.* [21] Given the important function of Tiam1 in cancer cell adhesion, migration, invasion and polarity, it will be interesting to analyze any potential Tiam1 function in neutrophils. Neutrophils also express members of the atypical (non Dbl-domain containing) DOCK GEFs, which are thought to be regulated by PIP_3 and also phosphatidic acid, and which can function as bipartite GEFs together with ELMO proteins. The analysis of DOCK2, DOCK5 and DOCK2/5-deficient neutrophils identified these DOCK family GEFs as important regulators of neutrophil polarization, chemotactic speed and persistent directionality.[22, 23] In an interesting twist, P-Rex1 has recently been shown to activate RhoG, which in turn regulates the DOCK2-ELMO complex to activate Rac signaling following GPCR activation of neutrophils.[24]

Whilst PI3K-regulated Rac activation is well established, more recently, PI3K was also been shown to drive Rac-inactivation.[25] Recently, the function of two PIP_3 activated Rac GAPs, ArhGAP15 and ArhGAP25, were analyzed in myeloid cells. ArhGAP15-deficient neutrophils chemotaxed with improved directionality *in vitro*, and were recruited more efficiently in a model of sepsis *in vivo*; they phagocytosed and killed pathogens more efficiently.[26] Phagocytosis in ArhGAP25 knock-down PLB-985 neutrophil-like cells or in monocyte-derived macrophages was mildly upregulated; ArhGAP25-deficient neutrophils exhibited reduced rolling but enhanced crawling under flow conditions. These neutrophils were also characterized by increased TEM and improved recruitment to sites of inflammation *in vivo*.[27, 28] These phenotypes are suggestive of a potential role of both of these GAPs in reducing host defense, perhaps in order to protect the host from neutrophil-inflicted damage.

Cdc42

Apart from its action on the actin cytoskeleton, Cdc42 is a well-established key regulator of polarization in many biological systems. Interestingly, in the neutrophil, Rac rather than Cdc42 was thought for some time to control polarity and directionality (reviewed in ref [10]). The function of Cdc42 function in these processes has since been analyzed in neutrophils from (conditional) knock-out mice. This established that neutrophil directionality and polarity were impaired both when Cdc42 was deleted, or when (due to the deletion of Cdc42GAP) too much Cdc42 activity was present.[29, 30] An important function for Cdc42 in neutrophil polarization and directionality is also supported by the recent analysis of the localized activities of Rac, Ras, Rho and Cdc42 in fMLF-stimulated neutrophil-like PLB-985 cells using fluorescence resonance energy transfer (FRET)-based biosensors.[31] Interestingly, Cdc42-GTP became more distinctly localized to the leading edge than Rac-GTP upon chemoattractant stimulation of neutrophil-like PLB-985 cells. Cdc42-GTP redistribution preceded cellular turning, suggestive of a role in polarization. At the same time, RhoA-GTP was excluded from the leading edge, confirming an older report that employed FRET imaging to demonstrate RhoA activity at the trailing end of chemotaxing neutrophil-like HL-60 cells.[32] Interestingly, this distribution was observed irrespective of PI3K activity, and of the existence of a chemoattractant gradient.

PI3K-dependent regulation of Cdc42

We recently identified an unusual immune complex-induced pro-apoptotic pathway, PI3Kβ/δ-Cdc42-Pak-Mek-Erk, that operates in human neutrophils.[8] In this context, immune complex-induced Cdc42 activation was dependent on PI3K. In contrast, GPCR stimulation with the bacterial peptide fMLF caused Cdc42 activation irrespective of PI3K inhibition. This suggested the existence of a (directly or indirectly) PIP_3-activated Cdc42 GEF in the neutrophil, that acts downstream of FcγR but not GPCR stimulation. Given that integrins and FcγRs are known to share downstream signaling pathways, the as-yet-to-be-identified Cdc42 GEF is likely to function in adhesion-dependent situations as well. This observation is interesting, as there are only few instances in which Cdc42 has been shown to be activated in a PI3K-dependent fashion in any tissues, but for example, EGF-induced activation of Cdc42 in MTLn3 carcinoma cells was shown to be abrogated on inhibition of PI3K.[33] No Cdc42 GEF has yet been shown to be directly activated (or recruited to the plasma membrane) by PIP_3, but α-PIX was suggested to be involved indirectly in Gβγ, Pak, Cdc42 and PIP_3 co-localization to the leading edge of chemotaxing neutrophils.[34]

Conversely, PI3K has been demonstrated to regulate Cdc42 inactivation. Hence, Cdc42 inactivation was shown to be regulated by PI3K in phagocytosing mouse macrophage-like RAW264.7 cells.[35] Three PIP_3-activated Rac/Cdc42 GAPs were recently identified in a knock-down based screen analyzing the regulation of phagocytosis in RAW264.7.[36] In addition, Cdc42GAP was isolated as a PIP_3-binding protein from neutrophils.[9] As discussed above, Cdc42GAP was since shown genetically to regulate neutrophil chemotaxis and recruitment, with Cdc42GAP-deficient neutrophils characterized by reduced directionality but increased speed and enhanced TEM. This was associated with altered adhesive (podosome-like) structures and MAPK signaling.[29]

RhoA

RhoA is another regulator of cell migration, which is best known for controling contractile actin cables (stress fibres), and stable focal adhesions, which anchor the cell to the substratum. Actin cables, along with focal adhesions (and indeed the shorter lived focal contacts), do not actually exist in neutrophils. Nonetheless, RhoA has long been regarded as another important regulator of neutrophil migration. Using FRET microscopy, RhoA-GTP was shown to localize to the leading edge and trailing end of many cell types during cell migration. Such experiments have not yet been reported with primary neutrophils. In neutrophil-like cell lines (HL-60 and PLB-985), however, RhoA-GTP was shown to be restricted to the trailing end.[31, 32] This distribution is thought to be regulated by a series of regulatory feed-back loops.[10, 31, 32] The major function of RhoA in neutrophil migration is therefore thought to lie in the regulation tail retraction, mediated by its effector ROCK. However, a recent report on RhoA knock-out neutrophils has cast doubts on this assumption. Rho-deficient neutrophils displayed enhanced neutrophil integrin activation and were characterized by increased random and directional migration *in vitro* and by enhanced recruitment to inflammatory sites *in vivo*.[37] This suggests that RhoA may in fact act as a negative regulator of neutrophil migration and activation.

PI3K-dependent regulation of RhoA

No PI3K-activated RhoA GEF has been reported yet in the neutrophil, but observations in other cell types support the existence of a PI3K-regulated Rho GEF. For example, in p110α-deficient or p110α kinase-dead endothelial cells, or upon PI3K inhibition in wild-type endothelial cells, RhoA activation was dramatically reduced, correlating with a migration and tail retraction defect.[38]

Our understanding of PI3K-driven RhoA inactivation in the neutrophil is greater than that of Rho activation. ARAP3, a dual GAP for RhoA and Arf6, was identified in a screen for PIP3 binding proteins from neutrophils.[9] In ARAP3-deficient neutrophils, or in knock-ins carrying a point mutation that uncoupled ARAP3 from the activation by PI3K, adhesion dependent neutrophil functions were upregulated due to increased β2 integrin affinities.[39, 40] ARAP3-deficient neutrophils were not only characterized by an integrin-dependent migration defect but also by a chemotactic directionality defect. In-line with the reported enhanced chemotaxis of RhoA-deficient neutrophils toward KC (a mouse homologue of human IL8), but not toward fMLF,[37] the chemotaxis defect of neutrophils in which ARAP3 had been uncoupled from activation by PI3K that was more pronounced with migration toward MIP2 (another mouse homologue of IL8) than fMLF.[39]

Regulation of PI3K by Rho family GTPases

Rho family small GTPases have been reported to promote PI3K in a number of contexts. GTP-bound Rac and Cdc42 were shown to interact with the p110β RBD, directly activating it in a manner akin to Ras-dependent activation of p110α, p110γ and p110δ.[6] By analyzing knock-in mice that harbored mutations which interfered with the regulatory input of either Gβγ or Rac/Cdc42, PI3Kβ was shown to be activated synergistically by these two classes of activators on concurrent GPCR and RTK stimulation in macrophages (whilst GPCR stimulation alone relied heavily on PI3Kγ). ROS production and adhesion / spreading assays were used as indirect read-outs of PI3K activity in neutrophils to demonstrate that both Rac/Cdc42 and Gβγ-dependent stimulation of p110β were required in addition to FcγR (or indeed integrin) activation to derive full adhesion-dependent activation of PI3Kβ (ref [41] and Fig 2). In a series of elegant experiments, the authors of this study demonstrated that Gβγ and Rac/Cdc42 activated p110β in a cooperative fashion. Interestingly, a paracrine positive feed-back loop operates in FcγR- (or integrin-) stimulated neutrophils to enable this.[42] Hence, stimulation of the FcγR / integrin not only causes activation of Rac/Cdc42 via as yet undefined GEF(s) but also induces the production of the neutrophilic lipid mediator leukotriene B4 (LTB4), which binds to its (G protein coupled) receptor, BLT1, thereby generating G protein βγ subunits, that bind to the p110β RBD, driving the synergistic activation of this PI3K together with phosphopeptide. Interestingly, GPCR stimulation (using fMLF) alone relied almost entirely on

PI3Kγ rather than PI3Kβ.[41] Whilst this was not addressed in the study, it seems likely that BLT1 ligation also leads to the activation of downstream Rac/Cdc42 GEF(s), perhaps in conjunction with the PI3K lipid product PIP_3, adding an additional level of cross-talk between PI3K and Rac/Cdc42.

In chemotaxing mouse neutrophils (and human neutrophil-like cells), Rho family GTPases and PI3K have been reported to regulate one another by employing feed-back loops. Using a combination of inhibitors and probes as well as expression of dominant negative / constitutively active small GTPase constructs in HL60 cells showed that PIP_3 polarization to the leading edge required actin polymerization and Rac activation (reviewed in ref [10]). Building on these earlier studies, generation and localization of the PI3K lipid product PIP_3 were examined by activity assays and monitored using a fluorescent probe in the analysis of DOCK2-deficient neutrophils. Interestingly, in the absence of DOCK2, which is recruited to the neutrophil's leading edge by PIP_3, PIP_3 was generated, but not subsequently polarized.[22] The authors showed that PIP_3 was generated by DOCK2-deficient neutrophils, but did not drive persistent signaling. DOCK2 was proposed to be involved in a feed-back loop involving PI3K, Rac and actin which stabilizes the PIP_3 signal at the leading edge. In a separate study, RhoA was shown to downregulate PI3K signaling at the trailing end of chemotaxing neutrophils by activating the PIP_3 phosphatase PTEN.[43]

Conclusions and future directions

The examples given above outline significant cross-talk between PI3K and Rho family small GTPases in the neutrophil. This ensures the smooth running of processes that are controlled by these signaling intermediates; key areas studied with mouse knock-out neutrophils were polarization and chemotaxis, as well as *in vivo* neutrophil recruitment. Such studies have shown that the loss of GEFs and GAPs that regulate cross-talk between PI3K and small GTPases (such as CDC42GAP,[29] ARAP3[40] and DOCK2[22]) causes significant chemotaxis and polarization defects. Yet loss of function studies in the PI3K field have arrived at the conclusion that PI3K is dispensible for chemotaxis (reviewed in ref [5]). Could the role of PI3K have been underestimated? Recent findings with patients harboring rare PI3K gain of function mutations provided evidence that too much PI3K signaling can have devastating effects on lymphocyte function (e.g. ref [44]). Such a view is supported by the fact that increased PI3K signaling, due to the absence of the PIP_3 phosphatase SHIP1 severely affected neutrophil chemotaxis and polarization.[45] SHIP1-deficient neutrophils were characterized by poor migration and enhanced spreading; they failed to polarize PIP_3 at the leading edge of neutrophils. This raises the question whether cross-talk between PI3K and small GTPases, perhaps not only downstream of GPCR signaling, could be involved in correct PIP_3 (and neutrophil) polarization. This field promises to deliver further interesting insights in the future.

Key Words: neutrophil, chemotaxis, polarization, phagocytosis, NADPH oxidase, GPCR, FcγR, RhoA, Rac, Cdc42, PI3K, PtdIns(3,4,5)P_3

Abbreviations: PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; PIP_3, phosphatidylinositol-(3,4,5)-trisphosphate; GPCR, G protein coupled receptor; RTK, receptor tyrosine kinase; RBD, Ras binding domain; fMLF, N-Formyl-methionyl-leucyl-phenylalanine; FRET, fluorescence resonance energy transfer; TEM, transendothelial migration; LTB4, leukotriene B4; MPO, myeloperoxidase; NET, neutrophil extracellular trap.

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Figure Legends

Figure 1. Neutrophil function involves numerous processes that are regulated by Rho GTPases. Circulating neutrophils (centre) leave the circulation and chemotax along gradients of chemokines/chemoattractants to reach inflammatory sites (top left). In contrast to the round, circulating cells, chemotaxing neutrophils are polarized and characterized by a leading edge with actin-rich lamellipodium (indicated here by a zigzag line) and a trailing end. As professional phagocytes, neutrophils recognize and engulf small pathogens (e.g. bacteria and yeast; left). Phagocytosis involves the formation of a phagocytic cup which closes around the particle, forming the phagosome. Once engulfed, pathogens are killed intracellularly, in a process that employs ROS, antimicrobial peptides and proteases. Killing depends on two distinct processes, the assembly and activation of the NADPH oxidase (bottom left), as well as degranulation (bottom right). The NADPH oxidase catalyzes the generation of oxygen radicals, whilst degranulation ensures delivery of enzymes required for their conversion to biocidal ROS [in particular myeloperoxidase (MPO), a component of primary/azurophil granules; shown here in yellow]. Secondary/specific granules (shown in orange) deliver antimicrobial peptides and proteases into the phagosomes, which also contribute to intracellular killing. Under certain conditions killing occurs extracellularly, for example when pathogens are too large to be engulfed (e.g. parasites or fungal hyphae) or in conditions of sepsis, neutrophils release NETs (right). NETs consist of decondensed chromatin and antimicrobial proteins, and trap and kill pathogens. At the end of their short lives, neutrophils undergo apoptosis (top right). To limit the inflammation generated, they display ‘eat-me’ signals, thus triggering their own uptake by pro-resolution macrophages in a process termed efferocytosis.

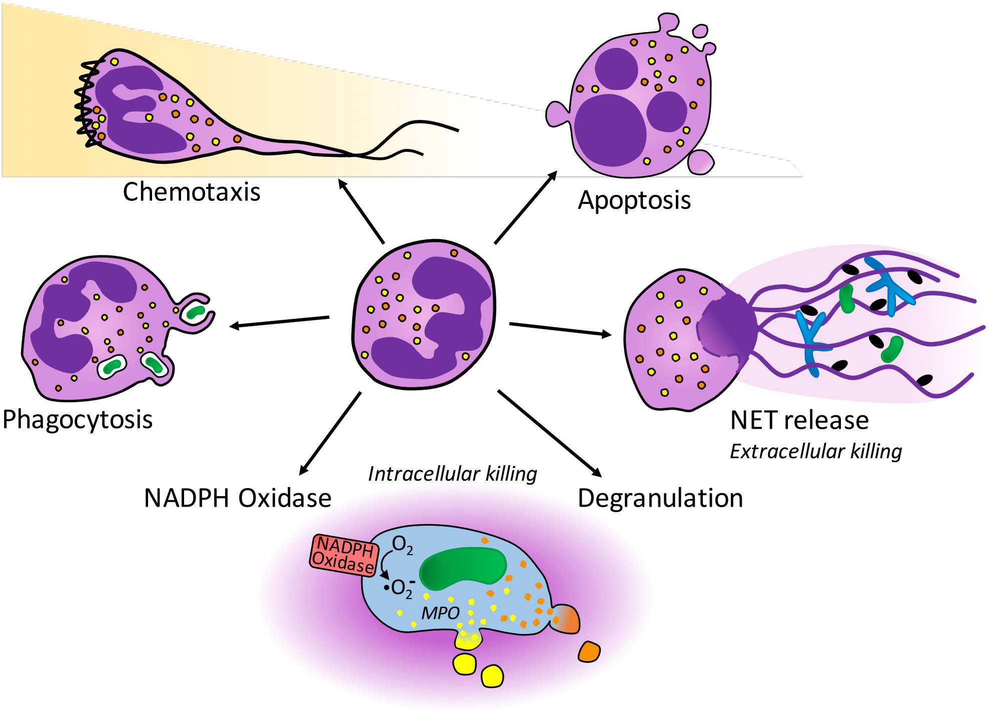
Figure 2. PI3Kβ is activated by phosphopeptide, Gβγ and Rac/Cdc42 in the integrin/immune complex-stimulated neutrophil. (Left) Neutrophil integrin or FcγR ligation

causes Src family kinase (SFK)-dependent phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs), triggering the activation of Syk kinase, which in turn recruits the p85 adapter in an SH2 domain- and phosphotyrosine motif-dependent fashion to activate PI3Kβ. Integrin/FcγR ligation and the PIP_3 also activate Rac/Cdc42 GEFs. (Right) In a paracrine feed-forward loop, PI3Kβ drives LTB4 production. LTB4 triggers activation of its GPCR (BLT1), resulting in release of Gβγ subunits activate PI3Kβ by binding to p110β. GPCR ligation and PIP_3 also activate Rac/Cdc42 GEFs. (Centre panel) Rac/Cdc42 GEFs enable GTP-loading of Rac/Cdc42, which can then bind the p110β RBD, to further activate PI3Kβ. Full activation of PI3Kβ requires phosphotyrosine motifs, Gβγ and Rac/Cdc42.

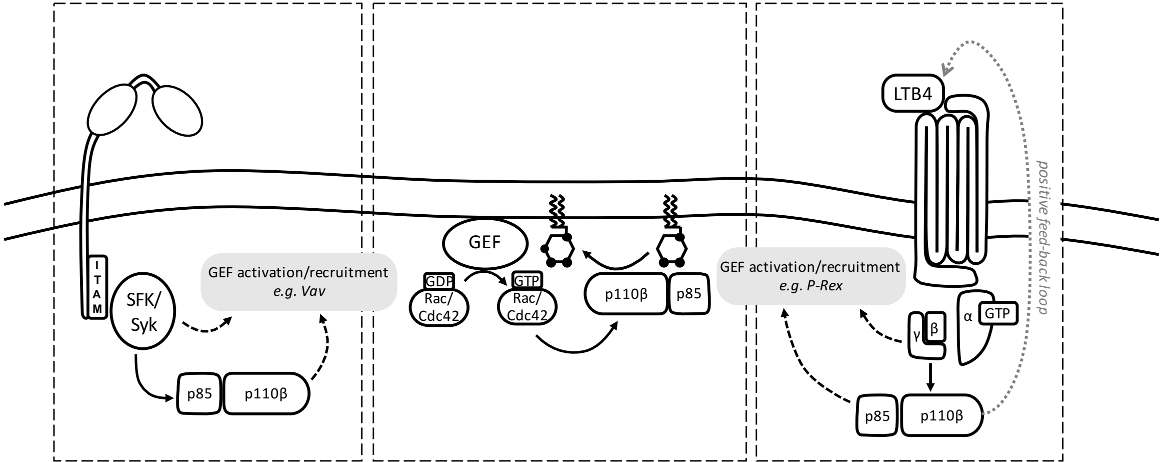
Neutrophil Function	Small GTPases involved	GEFs / GAPs involved
Adhesion	Rac2 (flow conditions) [11] RhoA (tail retraction [37])	Vav1/3 (static and flow conditions) [17] ArhGAP25 (flow conditions) [28] P-Rex1/Vav1 (static and flow conditions) [19] ARAP3 (static and flow conditions) [39, 40]
Spreading	Rac2 [11]	Vav1/3, Vav1-3 [17, 19] ARAP3 [39, 40]
Polarization	Cdc42 [30, 31] Rac2 [13] RhoG [24]	Cdc42GAP [29] DOCK2, DOCK2/5 [23, 24] ARAP3 [39]
Chemotaxis	Rac1 (directionality) [13] Rac 2 (migration and speed) [11, 13] Cdc42 [30, 31] Rho [37]	P-Rex1/Vav1 [19] DOCK2, DOCK5, DOCK2/5 [22, 23] ArhGAP15 (directionality) [26] Cdc42GAP [29] ARAP3 (migration and directionality) [39, 40]
Recruitment	Rac1 (sterile peritonitis) [12] Rac2 (sterile peritonitis) [11] Rho (acute lung injury) [37]	P-Rex1/Vav1 or -3 (to inflamed peritoneum or lung) [20] ArhGAP25 (sterile peritonitis) [28] ArhGAP15 (air pouch, bacterial peritonitis and abdominal sepsis) [26] Cdc42GAP (sterile peritonitis) [29] ARAP3 (sterile peritonitis and arthritic joint) [39]
Phagocytosis	Rac2 (Cdc42?)	Vav family (IgG and complement opsonized) e.g. [17] ARHGAP25 [27] ArhGAP15 (serum opsonized only) [26]
NADPH oxidase	Rac2 but not Rac1 e.g. [11, 12] RhoG (to fMLF and C5a) [15, 24]	Vav1-3 and ARHGAP25 (to opsonized particles) [19, 27] P-Rex1 (LPS-primed to fMLF) [19]

		P-Rex1/Vav1 (unprimed or primed to fMLF) [19] DOCK2, DOCK2/5 (to PMA) [23] ArhGAP15 (to fMLF or C5a but not opsonized zymosan) [26]
NET release	Rac2	DOCK2, DOCK5, DOCK2/5 [23]
Apoptosis	RhoG (in sterile peritonitis) [15] Cdc42 (immune complex induced apoptosis; primary human neutrophils) [8]	

Table 1. Regulation of neutrophil effector functions by Rho GTPases and their regulators. Many neutrophil functions are subject to regulation by Rho family GTPases. This table summarizes recent developments, mostly drawing from mouse models and placing a focus on GEFs and GAPs. Please note that due to space constraints not all papers could be cited.



Chemotaxis
Apoptosis
Phagocytosis
NET release
Extracellular killing
Intracellular killing
NADPH Oxidase
Degranulation
NADPH Oxidase
O_2
$\bullet O_2^-$
MPO



LTB4
positive feed-back loop
I
T
A
M
SFK/
Syk
GEF activation/recruitment
e.g. Vav
p85
p110β
GEF
GDP
Rac/
Cdc42
GTP
Rac/
Cdc42
p110β
p85
GEF activation/recruitment
e.g. P-Rex
α
GTP
γ
β
p85
p110β